

Assessment of feto-placental well-being in the mare from mid-gestation to term: Transrectal and transabdominal ultrasonographic features

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Abstract

Ultrasound assessment of feto-placental well-being has found its application in the equine field, as a valid diagnostic tool. Most reports on equine fetal parameters focus on advanced to late gestation. The aim of the present study was to further validate the technique of ultrasound evaluation of the equine fetal environment, by extending its application to earlier stages of pregnancy. Fetal parameters were collected (on 150 pregnancies) over a 3-year period. Data included in the study were selected according to the clinical behaviour of the mare, the neonate/fetus and fetal membranes at the termination of gestation. Validation of the ultrasound technique was also undertaken on a number of specimens collected at an abattoir. At the completion of the study, a substantial number of measurements and observations were collected. No fetal parameters associated in previous reports with a negative outcome were observed at any of the examinations during this study. Therefore, data collected during the present study should be of value in feto-placental assessment of well-being, in mid- to late-gestation.

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1. Introduction

Perinatal death still accounts for a large percentage of foal mortality, despite dramatic advances in neonatal intensive care medicine. Following the trend set in human perinatology, methods to assess equine feto-placental well-being during the latter stages of pregnancy, using both transabdominal [1–6] and transrectal ultrasonography [7,8], are being developed. The purpose of these techniques is to identify mares at risk of an abnormal pregnancy or delivery, thereby allowing closer supervision, during the periparturient period and earlier detection, treatment and possible prevention of costly neonatal disease.

The purpose of the present study was to further validate the technique of ultrasound assessment of equine feto-placental well-being, by extending its application throughout pregnancy, from day 150 of gestation to term. The study intended to establish reference guidelines of feto-placental development at different stages of pregnancy, based on ultrasound findings, as most reports in the literature focus on late gestation. This was accomplished by serial collection of transabdominal/transrectal ultrasound feto-placental parameters. Validation of the ultrasound technique was also undertaken on a number of specimens collected at an abattoir. This study was part of a larger equine reproductive project, aiming to assess: (1) the mare's reproductive tract prior to pregnancy, (2) hormone and ultrasound profiles during pregnancy, and (3) the neonate and fetal membranes at the termination of pregnancy.

2. Materials and methods

In the overall study, a total of 150 pregnancies (from 80 mares) were examined over a 3-year period. Pregnancies were classified, based on the clinical behaviour of the mare and the neonate/fetus and fetal membranes at the termination of gestation. The age of the mare and her past breeding history were known, but not used as classification criteria. Fetal parameters were included in this subsection of the study, when the pregnancies met the following criteria:

- (a) the mare produced a clinically normal, singleton foal, carried to term (≥ 320 day gestation);
- (b) the fetal membranes were spontaneously expelled within 90 min of foaling, not requiring manual extraction or oxytocin administration;
- (c) the foal stood and nursed the mare within 2 h of foaling;
- (d) the fetal membranes appeared grossly normal and intact after expulsion.

The study was conducted on a commercial standardbred farm, located in northern Italy. The mares were only housed at night, during the winter months. They were fed hay and concentrate feed, all year round. Mares were foaled indoors under close supervision. Time of foaling, time of fetal membrane expulsion, neonatal behaviour and vitality, and time to stand and nurse were all recorded. Inspection of the fetal membranes and clinical examination of all newborns was carried out within the first 24 hr of birth.

2.1. *Ultrasound examination*

Each examination involved both transrectal ultrasonography, using a B-mode, real time portable unit (Dynamic Imaging, Concept MCL, BCF Technology, Scotland), equipped with a 7.5-MHz linear transducer and transabdominal ultrasonography, performed using a sector scanner (Opus Plus, Ausonics, 1 Woodcock Place, Lane Cove, NSW 2066, Australia), with a choice of 2.5, 3.5, 5.0 and 7.5-MHz sector transducers. The mares were examined at monthly intervals from days 150 to 300 of gestation. Weekly ultrasound examinations were performed after day 300, until delivery.

The mares were restrained in purpose-built stocks, with both sides open, except for a single adjustable height bar. This allowed ease of access to both sides of the mare's ventral abdomen. Sedation was avoided, if possible, since this leads to suppression of fetal activity and heart rate reactivity [9–11]. To achieve optimal image quality, thorough preparation of the mare's ventral abdomen was obtained by clipping the hair with a No. 40 surgical blade, washing and liberal application of alcohol and ultrasound coupling gel. The clipped area extended from the mammary gland to the xyphoid and laterally to the level of the stifles. As pregnancy advanced, further clipping was done as required.

Transabdominal scans of the gravid uterus were initially performed with a 2.5 or 3.5-MHz sector scanner transducer, at a depth setting of 20–30 cm, as previously described [1–4,12]. For high-resolution images of the uteroplacental unit and smaller fetuses, a 5.0 or 7.5-MHz transducer was selected at an initial depth setting of 10–14 cm. The transducer and depth selected depended upon the size of the mare, the thickness of her ventral abdomen, the amount of fat or ventral edema present, and placental thickness. The pregnant uterus was scanned from the ventral abdomen, beginning just anterior to the mare's mammary gland and then moving cranially to determine fetal orientation. Transrectal scans of the gravid uterus visualise the most caudal portions of the equine fetoplacental unit. For this purpose, standard ultrasound assessment technique of the equine reproductive tract was used. All measurements recorded were expressed in millimetres. The duration of transabdominal scans varied from 30 to 90 min, depending on the mare's temperament and fetal activity. Ultrasound examinations were videotaped, using a Super-VHS video recorder and Super-VHS videotapes.

Fetal presentation, combined utero-placental thickness at the cervical pole, and orbital diameter of fetuses in anterior presentation, were assessed transrectally. Fetal activity and tone, heart rate, breathing movements, aortic diameter, uteroplacental thickness and contact, fetal fluid depth and quality, abdominal and thoracic fetal organs were evaluated by transabdominal scanning. Each fetal structure examined was repeatedly measured, on each session, at least three times, as described in the following sections.

2.2. *Fetal heart rate*

The fetal cardiac area was imaged within the thorax and multiple assessments of fetal heart rate and rhythm were made as described by Reef et al. [4,5]. Several fetal heart rates recordings, both at rest and after activity were obtained throughout the examination, using M-mode echocardiography. To do this, the fetal cardiac activity was identified, using two-dimensional B-mode ultrasonography and then an M-mode echocardiogram obtained by

placing the M-mode cursor through the moving fetal cardiac structures. Fetal heart rate was calculated using an in-built cardiac calculation package. The mean fetal heart rate for each examination was later calculated along with the maximum, minimum and range.

2.3. *Fetal aortic diameter*

The aortic diameter was measured in the thorax as it emerged from the heart, during systole, from leading edge to leading edge of the aortic wall, as described in previous studies [2–5]. It was distinguished from the vena cava by its echodense walls, pulsatile nature and its course within the thorax. As many as 10 measurements were obtained during each examination and the mean calculated.

2.4. *Activity*

Fetal activity was assessed during each examination and the type of movement recorded. Fetal tone was assessed as absent, if the fetus was inactive and appeared flaccid, or present, if the fetus flexed and extended the limbs, torso, neck and head and was able to perform complex movements. Different types of fetal activity were observed and identified as major and fine-tuned movements. Major movements include: whole body advancements within the uterus in cranio-caudal and/or ventro-dorsal directions and viceversa, rotation along the short and long axis. Fine-tuned activity episodes observed include: fetal head nods, flexion and extension of extremities, suckling motion, lip movements, blinking, nostril flaring, auricular pinnae motion, opening and closure of the glottis and tail movements. Only major movements were recorded in this study.

2.5. *Fetal breathing movements*

Fetal breathing activity was recorded when coordinated movements of diaphragm and ribs were detected, in the absence of fetal or maternal movement.

2.6. *Fetal orbit*

Once a clear image was obtained, two measurements of the orbit were made at right angles to each other and later summed.

2.7. *Trachea*

Measurements of the tracheal diameter were taken at the bifurcation. However, because it is anatomically flattened dorsoventrally in a C-shape, the diameter varied depending on whether it was measured on a frontal or cross-sectional plane.

2.8. *Stomach*

With the fetus in left lateral recumbency (LLR), within the gravid uterus and the scan performed in either the frontal or the cross-sectional plane, the stomach was closest to the

transducer (vice versa for a fetus lying in right lateral recumbency). The stomach was measured at its longest and widest points.

2.9. Kidney

Anatomically, the kidneys lie beneath the last couple of ribs and the right kidney is heart-shaped and lies more cranial than the left, which is bean-shaped. The length and width of the kidneys were measured at right angles to each other, when these organs were clearly identified.

2.10. Gonads

Measurements of the length and width of the gonads, which were observed caudal to the kidneys, along the thighs on frontal views, were taken at right angles to each other.

2.11. Fetal fluid depth, uteroplacental thickness and contact

The abdomen of the mare was divided into nine areas for the purpose of transabdominal determination of fetal fluid depth, uteroplacental thickness and contact: left caudal, mid-caudal, right caudal, left mid, mid-mid, right-mid, left cranial, mid-cranial and right cranial. Vertical measurements of fetal fluid and combined thickness of uterus and allantochorion were determined from scans, taken as perpendicular to the uteroplacental surface as possible. Areas of fetal contact were avoided during these measurements as compression of the uteroplacental unit was observed to occur.

2.12. CTUP: combined thickness of the utero-placental unit at the cervical pole

Transrectal measurements of the CTUP were obtained at the ventral aspect of the cervical pole, as described by Renaudin et al. [7]. Placental attachment was also evaluated in the region of the cervical star.

2.13. Validation

Validation of the ultrasound technique was carried out in the following manner. Intact gravid uteri, of known gestation, were collected at an abattoir and their contents scanned. Several fetal anatomical structures were identified and repeatedly measured, including: fetal orbit, trachea, cardiac shadow, stomach, kidney, and gonad. Once the scan was completed and the desired views and measurements of the fetal organs obtained, a necropsy was performed, using a standard Y-incision to expose the thoracic and abdominal cavities. The fetal eye, heart, trachea, stomach, kidney and gonad were dissected out from attached tissue and measurements (length, width and circumference), taken, using steel calipers and a measuring tape, for later comparison with those obtained using ultrasound.

Table 1

Fetal presentation (%) in mares from 6 to 12 month gestation (G6–G12)

Presentation ^a	G6	G7	G8	G9	G10	G11	G12
A	50	47	50	74	95	97.5	100
T	33	35	25	25	5	2.5	0
P	17	18	25	0	0	0	0

^a A: anterior; T: transverse; P: posterior.

3. Results: Data presentation and statistical analysis

For the purpose of this study the gestational age of the fetus was defined as follows:

Month of gestation: month 5 (G5): 120–150 day; month 6 (G6): 150–180 day; month 7 (G7): 180–210 day; month 8 (G8): 210–240 day; month 9 (G9): 240–270 day; month 10 (G10): 270–300 day; month 11 (G11): 300–330 day; month 12 (G12): 330–360 day; month 13 (G13): 360–390 day. Due to the size of the mares, the data collected from G5 were mainly obtained transrectally. Since the transrectal approach allowed at most a 5-min examination, data for G5 were not included in the present study.

The ultrasound results from both transrectal and transabdominal examinations were entered into Microsoft[®] Excel 98 (Microsoft International, Dublin, UK); the mean and S.D. were calculated for each parameter at each examination. Data were analysed using paired *t*-test, correlation coefficient or simple regression analysis where appropriate using Statview 5.0 (SAS Institute Inc., Cary, NC, USA). Data are summarized below in Tables 1–9.

4. Discussion

Fetal ultrasonography, using a combination of transrectal and transabdominal approaches, can visualise the equine fetus up to term. Fetal ultrasonography efficiently detected multiple fetuses, allowed fetal reduction up to mid-gestation, assessed fetal growth, activity, mobility, and presentation. Qualitative and quantitative assessment of fetal fluid, integrity of the utero-placental unit and combined thickness of uteroplacental

Table 2

Mean $\pm$ S.D. fetal heart rate at rest (HRR) and during activity (HRA) in mares from 6 to 12 month gestation (G6–G12)

Month	HRA	Max	Min	HRR	Max	Min
G6	130 $\pm$ 11.7	146	113	113 $\pm$ 9.0	120	100
G7	140 $\pm$ 19.8	176	115	112 $\pm$ 11.2	125	95
G8	124 $\pm$ 9.8	143	111	106 $\pm$ 6.2	115	95
G9	106.6 $\pm$ 9.6	120	90	91 $\pm$ 6.6	102	79
G10	110 $\pm$ 14.9	136	92	86.6 $\pm$ 7.9	98	82
G11	90.7 $\pm$ 16	140	70	72.5 $\pm$ 8	86	55
G12	86 $\pm$ 10.8	105	71	66.4 $\pm$ 6.5	77	56

Overlaps between HRA and HRR sometimes occurred, due to difficulties in recording HRA (especially in small fetuses).

Table 3

Mean of major fetal movements, recorded during 1 h of observation, in mares from 6 to 12 month gestation (G6–G12)

	Episodes/h
G6	5.95
G7	3.71
G8	2.49
G9	3.69
G10	3.66
G11	4.24
G12	3.16

Fetal activity was assessed as either present or absent and only major movements were recorded.

Table 4

Mean $\pm$ S.D. of fetal aortic diameter (AoD; mm) measurements, in mares from 6 to 12 month of gestation (G6–G12)

	AoD
G6	10.28 $\pm$ 1.7
G7	14.10 $\pm$ 1.6
G8	14.78 $\pm$ 1.5
G9	18.19 $\pm$ 1.6
G10	22.25 $\pm$ 1.5
G11	24.8 $\pm$ 1.4
G12	25.7 $\pm$ 1.05

unit at the cervical pole were all evaluated by ultrasound. Techniques of fetal monitoring, originally developed for the human fetus, have been adapted to the late-term equine fetus. The inability to accelerate equine fetal maturation and safely induce parturition represent a major limitation to the routine use of fetal monitoring techniques, to the same extent as they are applied in human medicine. The main clinical conditions, indicating the need for fetoplacental health assessment in the mare are: vaginal discharge, premature udder development, premature lactation, systemic illness, history of problematic pregnancies, sudden increase of abdominal volume, and surgery. Such conditions can occur at any stage of gestation, but the parameters available to assess fetoplacental wellbeing are limited to advanced gestation and term fetuses. An equine biophysical profile has been developed in recent years, adapted from a human model, using dynamic ultrasound techniques.

Table 5

Mean $\pm$ S.D. of combined fetal orbital diameters (FO; mm), in mares from 6 to 12 month of gestation (G6–G12)

	FO
G6	41.3 $\pm$ 2.0
G7	48.9 $\pm$ 1.0
G8	52.2 $\pm$ 4.6
G9	57.4 $\pm$ 4.9
G10	64.3 $\pm$ 2.9
G11	68.1 $\pm$ 3.6
G12	70.3 $\pm$ 2.7

Table 6

Mean $\pm$ S.D. combined uteroplacental thickness (CUPT; mm) assessed in various sites^a of the ventral abdomen in mares from 6 to 12 month of gestation (G6–G12)

	G6	G7	G8	G9	G10	G11	G12
LCD	7.08 $\pm$ 2.5	6.1 $\pm$ 2.0	7.29 $\pm$ 1.6	7.33 $\pm$ 2.23	7.67 $\pm$ 1.63	10 $\pm$ 5.14	10.1 $\pm$ 4.39
MCD	5.2 $\pm$ 1.04	6.57 $\pm$ 1.38	7.97 $\pm$ 2.34	8.07 $\pm$ 1.47	7.8 $\pm$ 2.0	9.01 $\pm$ 2.37	10.75 $\pm$ 5.9
RCD	5.6 $\pm$ 2.24	6.22 $\pm$ 1.65	7.8 $\pm$ 1.68	8.22 $\pm$ 1.86	7.22 $\pm$ 1.28	9.34 $\pm$ 2.91	9.5 $\pm$ 4.8
LM	5.6 $\pm$ 1.1	6.25 $\pm$ 1.61	6.98 $\pm$ 1.24	7.73 $\pm$ 2.79	8.66 $\pm$ 3.37	9.89 $\pm$ 4.01	10.7 $\pm$ 3.5
MM	5.1 $\pm$ 1.0	5.07 $\pm$ 1.49	6.46 $\pm$ 0.5	7.48 $\pm$ 2.1	8.7 $\pm$ 3.37	9.44 $\pm$ 3.37	11.1 $\pm$ 5.56
RM	5.17 $\pm$ 0.8	5.15 $\pm$ 1.55	7.58 $\pm$ 1.86	9.43 $\pm$ 5.6	8.7 $\pm$ 2.6	9.6 $\pm$ 3.81	8.88 $\pm$ 3.25
LCR	6.0	5.02 $\pm$ 1.11	5.98 $\pm$ 1.39	5.85 $\pm$ 1.69	7.6 $\pm$ 3.43	7.06 $\pm$ 2.73	7.58 $\pm$ 3.8
MCR	3.5	5.17 $\pm$ 1.65	7.84 $\pm$ 2.5	5.86 $\pm$ 1.15	6.7 $\pm$ 2.4	6.75 $\pm$ 2.3	5.95 $\pm$ 1.66
RCR	2.9	5.06 $\pm$ 0.8	6.01 $\pm$ 1.8	6.8 $\pm$ 2.5	7.2 $\pm$ 3.8	7.53 $\pm$ 2.99	6.38 $\pm$ 2.76
CX P	4.7 $\pm$ 0.2	5.0 $\pm$ 0.5	5.2 $\pm$ 0.8	7.7	8.3 $\pm$ 2.59	9.56 $\pm$ 2.42	15.7 $\pm$ 5.6

^a LCD: left caudal; MCD: mid-caudal; RCD: right caudal; LM: left-mid; MM: mid-mid; RM: right-mid; LCR: left cranial; MCR: mid cranial; RCR: right cranial; CX P: cervical pole.

Table 7

Mean $\pm$ S.D. fetal fluid depth measurements (FFD; mm) assessed in various sites^a of the ventral abdomen in mares from 6 to 12 month of gestation (G6–G12)

FFD	G6	G7	G8	G9	G10	G11	G12
LCD	38.0 $\pm$ 39.4	34.5 $\pm$ 35.5	49.1 $\pm$ 44.3	49.66 $\pm$ 33.11	62.4 $\pm$ 28.9	59.8 $\pm$ 41.7	47.6 $\pm$ 34.0
MCD	57.9 $\pm$ 35.2	22.8 $\pm$ 32.3	31.1 $\pm$ 23.1	45.96 $\pm$ 24.0	69.11 $\pm$ 41.79	50.0 $\pm$ 34.8	33.6 $\pm$ 28.5
RCD	57.0 $\pm$ 25.5	44.0 $\pm$ 41.6	26.0 $\pm$ 31.0	46.41 $\pm$ 30.5	48.38 $\pm$ 32.1	51.2 $\pm$ 30.3	48.8 $\pm$ 34.0
LM	20.4 $\pm$ 32.8	19.4 $\pm$ 18.3	56.5 $\pm$ 38.2	42.9 $\pm$ 19.0	50.3 $\pm$ 50.1	48.4 $\pm$ 33.9	47.1 $\pm$ 43.3
MM	61.5 $\pm$ 36.0	16.5 $\pm$ 22.5	23.9 $\pm$ 34.1	36.39 $\pm$ 14.0	52.5 $\pm$ 24.7	40.0 $\pm$ 25.8	26.4 $\pm$ 14.5
RM	64.5 $\pm$ 46.6	37.8 $\pm$ 50.8	14.9 $\pm$ 21.3	52.85 $\pm$ 26.3	36.75 $\pm$ 19.96	42.1 $\pm$ 18.3	45.3 $\pm$ 35.7
LCR	113.4 $\pm$ 66.0	51.6 $\pm$ 65.9	17.5 $\pm$ 18.7	41.93 $\pm$ 23.0	35.9 $\pm$ 27.3	27.8 $\pm$ 24.5	37.2 $\pm$ 40.3
MCR	69.0	14.5 $\pm$ 31.6	20.1 $\pm$ 26.1	37.7 $\pm$ 35.9	15.52 $\pm$ 2.43	35.8 $\pm$ 31.3	29.5 $\pm$ 24.6
RCR	17.3	15.8 $\pm$ 16.1	24.5 $\pm$ 48.1	44.6 $\pm$ 28.11	29.3 $\pm$ 13.19	33.4 $\pm$ 29.1	14.8 $\pm$ 3.7

^a LCD: left caudal quadrant; MCD: mid-caudal; RCD: right caudal; LM: left-mid; MM: mid-mid; RM: right-mid; LCR: left cranial; MCR: mid cranial; RCR: right cranial.

Reference values for different stages of gestation are needed to facilitate early detection of fetal malaise.

There is only one previous report of an attempt to validate the process of transabdominal ultrasound, using three dead fetuses [1]. The main purposes of the validation process in the present study were to establish the relationship between ultrasound and actual measurements of fetal organs. This was successfully accomplished and a positive correlation was found between both sets of measurements (Table 9). This was an important finding; unless there was a correlation between the two, it would cast doubt on the reliability of ultrasound to reflect actual fetal growth. Nevertheless, despite the strong

Table 8

Mean $\pm$ S.D. fetal stomach measurements (FS; mm) in mares from 6 to 12 month of gestation (G6–G12)

Axis ^a	G6	G7	G8	G9	G10	G11	G12
LA	59.3 $\pm$ 12.4	59.7 $\pm$ 9.4	75.3 $\pm$ 16	77.5 $\pm$ 11.1	97.6 $\pm$ 15	102.4 $\pm$ 8.7	98.8 $\pm$ 14.4
SA	33.2 $\pm$ 8.5	35.5 $\pm$ 6.1	43.8 $\pm$ 11	45.1 $\pm$ 9.8	58.7 $\pm$ 10.8	55.5 $\pm$ 8.7	57.1 $\pm$ 9.5

^a Long (LA) and short (SA) axis, respectively.

Table 9
Ultrasound-obtained and actual measurements (cm) obtained from equine fetuses ($n = 6$) at necropsy

	Fetus no. 1		Fetus no. 2		Fetus no. 3		Fetus no. 4		Fetus no. 5		Fetus no. 6	
Gender	Female		Male		Female		Female		Female		Female	
Gest. age (wk)	37		41		38		28		28		38	
Crown-rump length	85		96		84		63.5		59 cm		68 cm	
Thoracic width	24.5		30		27.5		—		—		—	
Orbital diameter*	3.3 ± 0.17		3.7 ± 0.18		3.6 ± 0.15		3.4 ± 0.12		3.2 ± 0.10		3.1 ± 0.15	
	3.6		3.8		3.65		3.6		3.5		3.2	
Cardiac shadow**	6.1 ± 1.2 × 4.4 ± 0.5		7.6 ± 0.29 × 5.5 ± 0.42 ^a		7.7 ± 0.33 × 5.0 ± 0 ^b		5.2 ± 0.06 × 3.58 ± 0.02 ^a		5.4 ± 0.12 × 3.9 ± 0.17 ^a		6.0 ± 0.4 × 4.5 ± 0.20 ^a	
			7.85 ± 5.29 ^b				4.8 ± 0.32 × 3.6 ± 0.26 ^b		5.3 ± 0.15 × 4.2 ± 0.25 ^b		6.5 ± 0.24 × 4.9 ± 0.27 ^b	
	7.5 ± 6.4		7.6 ± 6.6		8.2 × 6.5		5.4 × 4.0		5.8 × 4.3		7.6 × 4.8	
Trachea	1.3 ± 0.08		—		1.7 ± 0.02 ^a		—		0.8 ± 0.03 ^a		—	
	—		—		2.0 × 1.8		—		1.0 × 1.6		1.4 × 1.8	
Stomach**	5.9 ± 0.68 × 3.7 ± 0.5		—		6.3 ± 0.55 × 3.1 ± 0.29 ^a		7.5 ± 0.28 × 3.9 ± 0.26 ^b		5.7 ± 0.15 × 3.6 ± 0.2 ^a		6.9 ± 0.42 × 4 ± 0.38 ^a	
	—		—		10.0 × 7.5		10.6 × 7.0		6.5 ± 0.34 × 3.8 ± 0.40 ^b		6.9 ± 0.36 × 4.4 ± 0.2 ^b	
									10.7 × 6.5		11.6 × 6.9	
Kidney	—		—		3.6 × 3.6		—		—		—	
	—		10.4 × 7.4		8.7 × 6.5^c		—		6.6 × 5.9^c		8.2 × 5.2^d	
					8.5 × 7.0^d				7.9 × 4.8^d			
Gonad**	5.2 ± 0.29 × 3.1 ± 0.13		5.2 ± 0.25 × 3.1 ± 0.17 ^b		3.7 × 2.1 ^a		6.2 ± 0.32 × 3.6 ± 0.27 ^a		6.4 ± 0.30 × 3.4 ± 0.17 ^a		6.9 ± 0.42 × 4.0 ± 0.20 ^a	
					6.4 × 3.5 ^b		7.1 ± 0.29 × 3.5 ± 0.24 ^b		6.9 ± 0.54 × 3.6 ± 0.43 ^b		6.8 ± 0.39 × 4.0 ± 0.20 ^b	
	5.6 ± 0 × 3.8 ± 0.07		7.1 × 4.4		6.3 × 3.7		8.1 ± 0.14 × 5.0 ± 0		8.8 × 5.4		7.7 × 5.0	

Ultrasound measurements (mean ± S.D.); italics; actual measurements (mean ± S.D.); bold.

^a Fetus in right lateral recumbency.

^b Fetus in left lateral recumbency.

^c Right kidney.

^d Left kidney.

* Difference ($P < 0.05$) but positive correlation ($P < 0.05$) between ultrasound-obtained and actual measurements.

** Difference ($P < 0.0001$) but positive correlation ($P < 0.0001$) between ultrasound-obtained and actual measurements.

positive correlation, there were also significant differences between the ultrasound and actual measurements obtained (Table 9). This was not surprising; although every effort was made to insure that both sets of measurements were taken in a corresponding scan plane, sometimes these differed and discrepancies were observed to occur. The relationship between ultrasound measurements and actual fetal organ size is sufficient to allow the detection of trends in fetal growth.

In the present study, the parameters consistently examined for assessment of feto-placental well being were as follows: fetal presentation, fetal heart rate and rhythm, fetal activity, fetal size, stomach measurements, uteroplacental thickness and contact, including the cervical pole and fetal fluid depth. Over the course of each ultrasound assessment many other parameters were recorded, but not included in the study for lack of completeness.

4.1. Fetal presentation

Fetal presentation was defined as anterior, posterior or transverse, according to fetal orientation in relation to the mare's pelvic inlet. Frequent changes in fetal presentation have been observed in studies investigating the mobility of the equine fetus [13–15]. In the present study, variability of fetal presentation decreased as gestation advanced (Table 1). From day 270 to term, all presentations were recorded as anterior. Temporary transverse presentation was detected in two fetuses at 10 and 11 month gestation. This is in agreement with the findings of Ginther and Griffin [15], who have shown an equal likelihood of anterior or posterior presentation during the first 5 month, with an increased likelihood of anterior presentation thereafter. These findings suggest that detecting a fetus in posterior presentation in the last 2 month of gestation is highly unusual and that the fetus is likely to remain that way.

Changes in fetal presentation, during the course of a single examination were recorded as episodes of fetal activity.

4.2. EFBP: fetal heart rate (FHR) and rhythm

The healthy equine fetal cardiac rhythm is usually regular [4,16,17]. A basal FHR of 75 ± 7 per min has been reported by Reef, during late gestation, in Thoroughbred and Quarter horse equine fetuses, using M-mode echocardiography [4]. As in humans, episodes of FHR variability in equine fetuses (accelerations and decelerations), increase in frequency as gestation progresses and transient (15–30 s) accelerations usually coincide with periods of fetal activity [2]. The most vigorous fetal activity and exaggerated FHR responses reportedly occur 48–72 h prior to parturition [2].

Abnormal FHR patterns in the equine fetus include persistent fetal tachycardia, bradycardia and cardiac arrhythmias. Fetal tachycardia has been observed preceding abortion [5,18] and stillbirth [2,5]. It has also been documented during abnormal deliveries [16]. Fetal bradycardia, inappropriate for gestational age, has also been detected [1,2,5,19] and was associated with a negative outcome. It is regarded, by some investigators, as the most reliable indicator of impending fetal demise [19]. Cardiac accelerations in response to fetal movement have been observed in equine fetuses using fetal electrocardiography [18], and Doppler transabdominal ultrasound [2,3,20]. Adams-Brendemuehl and Pipers [2],

found that spontaneous fetal activity generally resulted in accelerations of fetal heart rate of 25–40 beats per minute in amplitude and 20–40 s in duration. Equine fetal movements without FHR accelerations, were observed routinely, while accelerations in the absence of detectable stimuli occurred only 5% of the time [2,4].

Stress testing has also been performed in the pregnant mare by administration of oxytocin or the use of a vibroacoustic device placed in close proximity to the fetal head, during rectal palpation. Preliminary findings suggest that stress testing maybe of use in the assessment of fetal well-being, but further studies are needed to determine if it can be used as an effective means of discriminating between the normal and compromised equine fetus [10].

In the present study, fetal heart rates were recorded from 150 day of gestation to term (Table 2). This differs from previous studies, which have largely focussed on measurements of fetal heart rates during the last month of gestation and approaching term [2,4–6]. In the present study, the fetal cardiac rhythm was usually regular.

4.3. EFBP: fetal activity

The presence or absence of fetal movement is a reflection of central nervous system function, with depressed CNS function resulting in decreased activity. In late gestation, equine fetuses exhibit movements ranging from simple flexion and extension of extremities, to complex coordinated activities, including rotation of 360 degrees about the long axis [1,2,4,5]. Simple fetal movements were found to occur regularly throughout gestation from day 90 onwards, while complex movements were observed in the last 3 month of gestation. These movements are required to ensure satisfactory muscular development and the proper function of skeletal joints. The equine neonate is particularly dependent on the high level of development of these anatomical parts to ensure successful postnatal adaptation. Dormant (inactive) phases are observed in fetuses of all ages, but are more common in late gestation [21], when they usually last ≤ 10 min [4,21]. However, 30–60 min or longer dormant phases have been detected on occasion [21]. Therefore, caution should be exercised in interpreting fetal activity and reassessments are advisable. The equine fetus is usually very active and, long periods of true inactivity should be regarded as important (Table 3). Lack of fetal movement has been associated with a negative outcome in equine fetuses in a number of studies [1–3,5,22]. However, sudden bouts of excessive activity followed by abrupt cessation have also been recorded in fetuses that subsequently died [22]. Therefore, detection of either prolonged inactivity or hyperactivity during transabdominal ultrasonography is suggestive of a poor fetal outcome.

4.4. EFBP: fetal size

In the equine, fetal growth depends upon adequate placental development and in a normal pregnancy, fetal size and placental surface area are correlated [23,24]. As a result of the large size of the equine fetus, especially in the latter stages of gestation, imaging of anatomical structures for correlation of size and fetal age is limited to smaller structures that can be consistently imaged in their entirety. Structures that have been measured sequentially include fetal aortic diameter [2–6,22] and fetal orbital diameter [25,26].

Measurements of the fetal aorta were included in the biophysical profile developed by Adams-Brendemuehl and Pipers [2] and Reef et al. [5,6]. Fetal aortic diameter was significantly correlated with neonatal foal weight, girth and hip height in normal pregnancies [2,4]. Mean fetal aortic diameter of 2.28 ± 0.22 cm, have been reported by Reef et al. [4], for normal equine Thoroughbred and Standardbred fetuses, at the last scan prior to parturition. Reef et al. [5], found that the aortic diameter was a useful indicator of unfavourable intrauterine conditions for fetal growth. In addition it was consistently measurable and so was included in the biophysical profile for the equine fetus. In the present study, fetal aortic diameter measurements were easily determined and increased with advancing gestational age (Table 4).

The fetal orbit is accessible throughout gestation by either the transrectal or transabdominal route. McKinnon et al. [26] calculated orbital dimensions from the sum of the length and width of the orbit. However, since they obtained 95% confidence intervals of ± 0.5 cm (or ± 40 day), fetal orbit size was considered an insensitive estimator of fetal age. Approximate eye volume is the eye parameter that gives best assessment of fetal growth [27], but it has not been found to be significantly correlated with foal birth weight. In the present study, the fetal orbit was available for measurements over the entire course of gestation from day 150 to term, using transrectal ultrasound, when the fetus was in anterior presentation. As expected, there was a significant relationship between fetal orbital size and gestational age (Table 5), with orbital size increasing as gestation advances. Fetal orbit was found to offer a rough estimate of trends in fetal growth.

4.5. Stomach measurements

The hypoechoic, bean-shaped outline of the fetal stomach represents an easy landmark for fetal orientation. Its location within the fetal abdomen allows identification of left and right lateral fetal recumbency (within the mare's abdomen). Recording variations of lateral recumbency will recognise episodes of activity, manifest as rotations over the long axis. The size of the fetal stomach increases as gestation advances (Table 8). A direct correlation between stomach size and fetal swallowing activity has been demonstrated [30].

4.6. Fetal breathing movements

According to a study by Reef et al. [4], rhythmical breathing movements were observed in all fetuses in which the diaphragm could be visualised. Nevertheless, the assessment of fetal breathing movements was not included in Reef's [5] equine fetal biophysical profile, probably because they could not consistently be evaluated. In the present study, fetal breathing movements were occasionally observed, as excursions of the diaphragm between thorax and abdomen and expansion of the ribcage, from 8 month gestation to term.

4.7. EFBP: uteroplacental thickness and contact

For appropriate exchange of blood, nutrients, oxygen and wastes, uteroplacental contact must be sufficiently complete. The need for adequate uteroplacental contact area in the

mare is highlighted by the difficulty mares have in maintaining pregnancies involving twins [28]. However, small areas of separation of the placental membranes and uterus have been observed in some normal pregnancies [4,29] without any apparent effect on the health of the fetus.

An average combined thickness of the uteroplacental unit of 1.26 ± 0.33 cm has been reported in mares with normal pregnancies in late gestation, based on vertical measurements recorded from transverse scans along sagittal or parasagittal planes on the mare's ventral abdomen [2]. Reef et al. [4], suggested to obtain measurements, avoiding areas of compression of the uteroplacental thickness by the fetus. A significant relationship was not found between uteroplacental thickness and either gestational age or postpartum placental weight [2].

The thickness of the uteroplacental unit is affected by numerous factors, which may reduce the efficiency of placental function. Such conditions include placentitis, placental edema and premature placental separation. A thickened uteroplacental unit, as a result of placentitis, has been associated with a negative fetal outcome in a study by Adams-Brendemuehl and Pipers [2]. Large and/or progressively enlarging areas of placental separation, as observed by Reef et al. [5], may lead to inefficient exchange of nutrients and adversely affect fetal well-being. In addition to placental thickening, an abnormally thin uteroplacental unit has also been associated with a poor fetal outcome [5].

Since only the parts of the uterus and placenta in contact with the ventral abdominal wall can be examined (using transabdominal ultrasound), focal areas of placental abnormality may be missed. The method of transabdominal uteroplacental measurement is unique to this study, in which measurements were obtained from nine areas in relation to the mare's abdomen. Consequently, it is difficult to compare these results with those of studies by Adams-Brendemuehl and Pipers [2], Reef et al. [4–6], Renaudin et al. [7]. Uteroplacental contact was complete in the present study, although small areas of separation of placenta and uterus have previously been observed in normal mares [4,29], without apparent adverse effects on the fetus. In the present study, the ultrasound appearance of the uteroplacental unit was normal. Placental thickness measurements were found to vary, depending on the location assessed (Table 6). The nonpregnant horn had a folded, thickened appearance and, although normal, could lead to a false interpretation of uteroplacental thickening, whereas the pregnant horn could appear falsely thin, depending on whether or not the fetus was in contact with it.

4.8. *EFBP: fetal fluid depth*

The equine pregnancy includes both an allantois and amnion that are filled with fluid. Allantoic fluid predominates during both early and late gestation and measures 8–15 L at term. In contrast, the amniotic fluid is low during the first 3 month of gestation, then increases more rapidly to equal the volume of allantoic fluid during mid-pregnancy. At term, it measures 3–5 L [31,32]. Maximal allantoic and amniotic fluid depth measurements were included in the biophysical profile proposed by Reef et al. [5].

Pathological increases in amniotic fluid have been reported [33–36]. Markedly reduced amounts of amniotic fluid have also been observed in mares with severe systemic illness

[23]. Excessive accumulation of allantoic fluid has been recognised in several instances [23,33,37,38]. Reduced amounts of allantoic fluid have also been observed in mares associated with a poor fetal outcome. In the present study, the total fetal fluid depth was not significantly related to gestational age (Table 7). Total fetal fluid depth was measured in the same nine areas as described for the uteroplacental thickness and the two parameters were recorded simultaneously. Distribution of fetal fluids was directly related to positioning of the fetus within the uterus. No preferential area of maximal fluid depth could therefore be detected in the present study.

4.9. Transrectal measurement of the cervical pole placenta

Normal ranges for the combined thickness of the uterus and placenta (CTUP), at an area immediately cranial to the cervix have been established from 4 month gestation to term in normal pregnant mares using transrectal ultrasound [7,39]. The mean CTUP was found to plateau at approximately 4 mm between the 4th and the 9th month of pregnancy, after which time it increased significantly by 1.5–2 mm each month until the end of gestation. Troedsson et al. [8], suggested that under field conditions, CTUP of up to 7 mm prior to day 300 could be considered normal. Measurements should be obtained from a consistent area on the ventral body of the uteroplacental unit. A CTUP within 2S.D. of the controls established by Renaudin et al. [7], has been associated with normal pregnancies. Cases of ascending placentitis have been detected by a marked increase in CTUP, measured on ultrasound, and confirmed on histopathological examination [40].

In the present study, considerable variation in the ventral CTUP was observed among examinations in different mares (Table 6). A consistent increase in the mean ventral CTUP, from month to month, was recorded. Fetal fluid dynamics and positioning of the fetus will dictate the state of distension of the UP unit at the cervical pole. Fetal limbs and/or skull are often observed, within the mare's pelvis, pressing against the cervical pole. Caution should therefore be exercised in interpreting transrectal scans at all stages of gestation, unless the placental thickening is noticeably pronounced. Cervical pole edema was occasionally observed, in this study, during the last month prior to foaling.

4.10. Echogenicity of fetal fluids

Free floating particles (vernix) were detectable with ultrasound within both fetal fluid compartments from mid-gestation to term in the equine pregnancy.

Amniotic fluid has been described to originate from amniotic epithelium, fetal urine (in early gestation), saliva and secretions of the nasopharynx of the fetus [41]. As pregnancy advances, the equine fetal skin releases vernix, which increases the cloudiness of the amniotic fluid [42,43]. The free-floating particles found in allantoic fluid may reflect fetal kidney function. Fetal urine passes through the urachus and is stored in the allantoic cavity, resulting in high concentrations of calcium in the allantoic fluid [41,44]. The echogenicity of fetal fluid increases during episodes of fetal activity, as sedimented particles are actively stirred around by fetal parts in motion; this accounts for the increased echogenicity of amniotic versus allantoic fluid previously reported [7,39].

5. Conclusions

In conclusion, the measurements and observations collected in this study should be of value in fetoplacental assessment of well-being, in mid- to late-gestation. It is interesting to note that the fetoplacental parameters associated in previous studies with a negative outcome were not observed during this study.

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